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Vascular-specific growth factors and blood vessel formation.

Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J.

Regeneron Pharmaceuticals, Inc., Tarrytown, New York 10591, USA.

A recent explosion in newly discovered vascular growth factors has coincided with exploitation of powerful new genetic approaches for studying vascular development. An emerging rule is that all of these factors must be used in perf harmony to form functional vessels. These new findings also demand re-evaluation of therapeutic efforts aimed at regulating blood vessel growth in ischaemia, cancer and other pathological settings.

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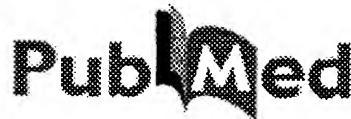
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Division of Neurology Research, Children's Hospital of Philadelphia, Pennsylvania 19104-4318, USA.

By screening a human fetal brain cDNA library under low stringency using cDNA encoding the mouse ligand of Cek5 as a probe, we have isolated a novel cDNA belonging to the EPLG gene family. This family encodes ligands of EPH-related tyrosine kinase receptors. Since the novel gene is the eighth member of the EPLG gene family, it is designated EPLG8. The deduced amino acid sequence of EPLG8 suggests that it encodes a transmembrane protein that is most related to those encoded by EPLG2 and EPLG5. We mapped the EPLG8 gene to human chromosome 17p11.2-p13.1 by PCR screening of human-rodent somatic cell hybrid panels. In the midterm fetus, EPLG8 mRNA is expressed at the highest level in brain, followed by heart, kidney, and lung. In the adult, EPLG8 mRNA expression is restricted to brain. These data suggest that LERK-8, the protein encoded by EPLG8, is important in brain development as well as in its maintenance. Moreover, since levels of EPLG8 expression were particularly high in several forebrain subregions compared to other brain subregions, LERK-8 may play a pivotal role in forebrain function.

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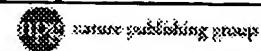
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Ephrin-B3, a ligand for the receptor EphB3, expressed at the midline of the developing neural tube.

Bergemann AD, Zhang L, Chiang MK, Brambilla R, Klein R, Flanagan J^t

Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA.

The ephrins are a family of ligands that bind to Eph family receptor tyrosine kinases, and have been implicated in axon guidance and other patterning processes during vertebrate development. We describe here the identification and characterization of murine ephrin-B3. The cDNA encodes a 340 amino acid transmembrane molecule, most closely related to the two other known transmembrane ligands, ephrin-B1 and ephrin-B2. In addition to homology in their extracellular receptor binding domains, these transmembrane ligands share striking homology between their cytoplasmic domains, with 31 of the last 34 amino acids of ephrin-B3 being identical to ephrin-B2, suggesting functional interactions of the cytoplasmic tail. While most Eph family ligands are promiscuous in their interactions with Eph receptors, binding studies with the receptors known to bind other transmembrane ligands only revealed a high affinity interaction of ephrin-B3 with EphB3, with a dissociation constant of approximately 1 nM. In situ hybridization of mouse embryos showed ephrin-B3 is expressed prominently at the dorsal and ventral midline of the neural tube, particularly in the floor plate, a structure with key functions in patterning the nervous system. The isolation of this ligand may help to elucidate the molecular basis of patterning activities at the neural tube midline.

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Induction of Eph B3 after spinal cord injury.

Miranda JD, White LA, Marcillo AE, Willson CA, Jagid J, Whittemore S.

Department of Neurological Surgery, University of Miami School of Medicine
1600 Northwest 10th Avenue, R-48, Miami, Florida 33136, USA.

Spinal cord injury (SCI) in adult rats initiates a cascade of events producing a nonpermissive environment for axonal regeneration. This nonfavorable environment could be due to the expression of repulsive factors. The Eph receptor protein tyrosine kinases and their respective ligands (ephrins) are families of molecules that play a major role in axonal pathfinding and target recognition during central nervous system (CNS) development. Their mechanism of action is mediated by repellent forces between receptor and ligand. The possible role that these molecules play after CNS trauma is unknown. We hypothesized that an increase in the expression of Eph proteins and/or ephrins may be one of the molecular cues that restrict axonal regeneration after SCI. Rats received a contusive SCI at T10 and in situ hybridization studies 7 days posttrauma demonstrated: (i) a marked up-regulation of Eph B3 mRNA in cells located in white matter at the lesion epicenter, but not rostral or caudal to the injury site, (ii) an increase in Eph B3 mRNA in neurons in the ventral horn and intermediate zone of the gray matter, rostral and caudal to the lesion. Immunohistochemical analyses localizing Eph B3 protein were consistent with the mRNA results. Colocalization studies performed in injured animals demonstrated increased Eph B3 expression in white matter astrocytes and motor neurons of the gray matter. These results suggest that Eph B3 may contribute to the unfavorable environment for axonal regeneration after SCI. Copyright 1999 Academic Press.

PMID: 10192794 [PubMed - indexed for MEDLINE]

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Ephrin-B3 is the midline barrier that prevents corticospinal tract axons from recrossing, allowing for unilateral motor control.

Kullander K, Croll SD, Zimmer M, Pan L, McClain J, Hughes V, Zabski J, DeChiara TM, Klein R, Yancopoulos GD, Gale NW.

European Molecular Biology Laboratory, D-69117 Heidelberg, Germany.

Growing axons follow highly stereotypical pathways, guided by a variety of attractive and repulsive cues, before establishing specific connections with distal targets. A particularly well-known example that illustrates the complexity of axonal migration pathways involves the axonal projections of motor neurons located in the motor cortex. These projections take a complex route during which they first cross the midline, then form the corticospinal tract, and ultimately connect with motor neurons in the contralateral side of the spinal cord. These obligatory contralateral connections account for why one side of the brain controls movement on the opposing side of the body. The netrins and slits provide well-known midline signals that regulate axonal crossings at the midline. Here we report that a member of the ephrin family, ephrin-B3, also plays a key role at the midline to regulate axonal crossing. In particular, we show that ephrin-B3 acts as the midline barrier that prevents corticospinal tract projections from recrossing when they enter the spinal gray matter. We report that in ephrin-B3(-/-) mice, corticospinal tract projections freely recross in the spinal gray matter, such that the motor cortex on one side of the brain now provides bilateral input to the spinal cord. This neuroanatomical abnormality in ephrin-B3(-/-) mice correlates with loss of unilateral motor control, yielding mice that simultaneously move their right and left limbs and thus have a peculiar hopping gait quite unlike the alternate step gait displayed by normal mice. The corticospinal and walking defects in ephrin-B3(-/-) mice resemble those recently reported for mice lacking the EphA4 receptor, which binds ephrin-B3 as well as other ephrins, suggesting that the binding of EphA4-bearing axonal processes to ephrin-B3 at the midline provides the repulsive signal that prevents corticospinal tract projections from recrossing the midline in the developing spinal cord.

PMID: 11297511 [PubMed - indexed for MEDLINE]

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Ephrin-B3-EphA4 interactions regulate the growth of specific thalamocortical axon populations in vitro.

Takemoto M, Fukuda T, Sonoda R, Murakami F, Tanaka H, Yamamoto I

Neuroscience Laboratories, Graduate School of Frontier Biosciences, Osaka University, Toyonaka, Osaka 560-8531, Japan.

The role was studied of ephrin-B3, a ligand of the Eph family of tyrosine kinase receptors, in the formation of cortical connectivity. *In situ* hybridization and immunohistochemistry showed that EphA4, a receptor of ephrin-B3, was expressed in the lateral thalamus (visual and somatosensory thalamus) of the developing rat brain, but not in the medial thalamic nuclei which project to the limbic cortex. Correspondingly, ephrin-B3 was expressed strongly in the developing limbic cortex including amygdala, entorhinal cortex and hippocampus. To examine the action of ephrin-B3 on thalamic axons, either lateral or medial thalamic explants were cultured on membranes obtained from ephrin-B3-expressing COS cells. Axonal growth was inhibited for cells from the lateral thalamus but not from the medial thalamus. These results suggest that ephrin-B3 contributes to regional specificity by suppressing axonal growth of lateral thalamic neurons.

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Mouse ephrinB3 augments T-cell signaling and responses to T-cell receptor ligation.

Yu G, Luo H, Wu Y, Wu J.

Laboratory of Immunology and the Nephrology Service of Notre Dame Hospital Centre Hospitalier de l'Universite de Montreal, Universite de Montreal, Montreal, Quebec H2L 4M1, Canada.

Ephrins (EFN) are cell-surface ligands of Ephs, the largest family of cell-surface receptor tyrosine kinases. The function of EFNs in the immune system has not been well studied, although some EFNs and Ephs are expressed at high levels in certain leukocytes. We report here that EFNB3 and its receptors (collectively called EFNB3Rs, as EFNB3 binds to multiple EphBs) were expressed in peripheral T cells and monocytes/macrophages, with T cells being the dominant EFNB3+ and EFNB3R+ cell type. Solid-phase EFNB3-Fc in the presence of suboptimal anti-CD3 crosslinking enhanced T-cell responses in terms of proliferation, activation marker expression, interferon-gamma but not interleukin-2 production, and cytotoxic T-cell activity. EFNB3R costimulation in the presence of phorbol 12-myristate 13-acetate was insensitive to cyclosporin A, similar to CD28 costimulation, suggesting they might share a part of the signal pathway. After crosslinking, T-cell receptor and EFNB3R congregated into aggregated rafts, and this provided a morphological basis for signaling pathways of T-cell receptor and EFNB3R to interact. Solid-phase EFNB3-Fc augmented p38 and p44/42 MAPK activation further downstream of the signaling pathway. These data suggest that EFNB3 is important in T-cell/T-cell and T-cell/antigen-presenting cell collaboration to enhance T-cell activation and function.

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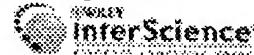
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EphA4 provides repulsive signals to developing cochlear ganglion neurites mediated through ephrin-B2 and -B3.

Brors D, Bodmer D, Pak K, Aletsee C, Schafers M, Dazert S, Ryan AF.

Department of Surgery, Division Otolaryngology and Neurosciences, University of California, San Diego School of Medicine and Veterans Administration Medical Center, La Jolla 92093, USA.

The ephrins and Eph receptors make up two large families of bi-directional signaling molecules that are known to play a role in the development of the nervous system. Recently, expression of EphA4 in the developing cochlea was shown, with strong expression in cells lining the osseous spiral lamina (OSL) through which afferent dendrites must pass to reach the organ of Corti (OC). It was also demonstrated that ephrin-B2 and -B3, both of which are known to interact with EphA4, are expressed by spiral ganglion (SG) neurons. To investigate the functional role of EphA4 in the development of inner ear neuro neonatal rat SG explants were cultured for 72 hours on uniformly coated surface or near stripes of EphA4/IgG-Fc-chimera. Control explants were cultured on or near IgG-Fc and EphA1/IgG-Fc-chimera. To assess the roles of ephrin-B2 and B3 in EphA4 signaling, SG explants were cultured with or without anti-ephrin and/or -B3 blocking antibodies. Growth patterns of SG neurites at the border of EphA4 receptor stripes showed repulsion, characterized by turning, stopping and/or reversal. In the case of IgG-Fc and EphA1, the neurites grew straight or near the stripes. Treatment with either anti-ephrin-B2 or -B3 blocking antibodies significantly reduced the repulsive effect of an EphA4 stripe. Moreover, when both antibodies were used together, neurites crossed onto EphA4 stripes with no evidence of repulsion. The results suggest that EphA4 provides repulsive signals to SG neurites in the developing cochlea, and that ephrin-B2 and -B3 together mediate this response. Copyright 2003 Wiley-Liss, Inc.

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